RAPID DIAGNOSTICS

Diagnosis of gonococcal infection in high risk women using a rapid test

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Objective: To assess the performance and acceptability for patients and health care workers of the NGThermo Biostar (GC OIA) to diagnose gonococcal infection compared with culture using modified Thayer Martin medium.

Methods: This study involved 326 high-risk women presenting with vaginal discharge or referral by sexual partner with urethral discharge at a sexually transmitted infections (STI) clinic in Manaus, Brazil. Endocervical swabs collected from the women were tested with both the NG Biostar and modified Thayer Martin culture as the reference standard test. Clinic staff were trained to perform the NG Biostar on site and the culture was performed in the laboratory of the clinic.

Results: The prevalence of gonococcal infection as measured by the reference standard was 15% (50/326) overall. Among asymptomatic participants, the prevalence of infection was 17.7% (25/141) and among symptomatic women it was 13.5% (25/185) (p = 0.3). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the NG Biostar test, with 95% confidence intervals (CI), were 60% (46.4% to 73.6%), 89.9% (86.2% to 93.6%), 55.6% (42.4% to 68.8%), and 92.6% (89.5% to 95.7%), respectively; 98.8% of study participants were willing to wait approximately 1 hour in the clinic for test results.

Conclusion: Syndromic management protocols for treatment of STI in developing countries require refinement because, as currently described, they lead to over-treatment of cervical infection. A rapid test done during patients' initial presentation and leading to immediate treatment if positive would help improve the accuracy of diagnosis and could also be used to screen asymptomatic women. Even though the NG Biostar had a low sensitivity and PPV, which is less than ideal, it could still improve the rates of treatment over the gold standard test that requires return visits for patients to receive results and to benefit from treatment. Cost-effectiveness studies using rapid point-of-care tests for *Neisseria gonorrhoeae* infection compared to the syndromic approach should be carried out to assess their value in STI diagnosis and treatment in developing nations.

Gonococcal and chlamydial infections are among the most common bacterial sexually transmitted infections (STIs) worldwide. The World Health Organization (WHO) estimates that more than 62 million cases of gonorrhoea occur each year, with high rates consistently seen in developing countries.[1] Although there is no reliable countrywide data on gonococcal infection, based on regional studies and STI transmission rates, Brazil has a high incidence of gonorrhoea.[2] A study of sex workers in the Amazon region found a prevalence of gonococcal infection of 16.3%.[3] Infection by *Neisseria gonorrhoeae* is usually located in the mucosa or the cervix, urethra, rectum, and throat, though it is often asymptomatic in females.[4 5] Untreated gonorrhoea in women can cause pelvic inflammatory disease, leading to ectopic pregnancy, tubal infertility, and chronic pelvic pain, among other serious symptoms. In both sexes, disseminated gonococcal infection can lead to arthritis, meningitis, and endocarditis. Infected mothers can transmit *N gonorrhoeae* to neonates at birth, leading to ophthalmia neonatorum, a potentially blinding condition.

The WHO recommends the use of syndromic management as the most efficient option for STI cases in developing countries. The syndromic approach uses easily recognised signs and symptoms to choose a treatment that will cover the majority of organisms responsible for causing the possible syndrome, leading to immediate treatment of transmissible infections.[6] This approach does not work well for the syndrome of vaginal discharge, because vaginitis is a more common cause of the syndrome than cervical infection with *N gonorrhoeae* or *Chlamydia trachomatis*.[7 8] Syndromic management therefore results in over-treatment of *N gonorrhoeae* and *C trachomatis*.[6 8 9] The syndromic approach is more useful in men, where urethral discharge is more closely related to gonococcal and chlamydial infection.[9] Syndromic management is not applicable to the majority of gonococcal infections that are asymptomatic, which can only be detected by screening tests.[10]

Culture of *N gonorrhoeae* on modified Thayer-Martin medium is the reference standard, as it has high sensitivity and specificity[9 11] Nucleic acid amplification tests (NAATs), which amplify sequences of DNA unique to *N gonorrhoeae*, are an alternative diagnostic method. NAATs are faster than culture, and do not require viable organisms.[12 13] Although inherently more sensitive than culture, not all commercially available NAATs for *N gonorrhoeae* are acceptably specific.[9] For male urethral discharge, the Gram stain is a simple alternative for *N gonorrhoeae* detection, but it is not recommended for female cervical infection diagnosis even in settings with well trained technicians. The ideal test for diagnosis of gonococcal infection in primary care settings must not require sophisticated laboratory equipment and complicated techniques, since they are often not available, and require patients to return for their test results. Culture and NAATs require highly trained laboratory staff and can be affected by specimen transport conditions.[13 14] Additionally, they are too expensive to be widely used in developing countries.[9]

Abbreviations: NAAT, nucleic acid amplification test; NPV, negative predictive value; PPV, positive predictive value; STI, sexually transmitted infections; WHO, World Health Organization

The objective of this study was to determine the performance and acceptability for patients and health care workers of the NGThermo Biostar (GC OIA) to diagnose gonococcal infection, compared with the current reference standard (culture using modified Thayer-Martin medium).

SUBJECTS AND METHODS

The study was approved by the National Ethical Committee (CONEP) in Brazil and the WHO research ethics review committee. It involved 326 women 18 years or older at an STI clinic in Manaus, Amazon, North Brazil, presenting with vaginal discharge or referred by a sexual partner with urethral discharge. Women who had been prescribed anti-gonoccocal antibiotics in the 3 weeks before study entry were excluded. Use of antibiotics for non-gonococcal infections 3 weeks prior was recorded in the data collection form, but was not an exclusion criterion. Women menstruating at presentation were excluded since the presence of blood affects the performance of the assay. The women who met all the criteria were asked to participate in the study. Those who gave written consent underwent an interview and physical examination according to routine clinic protocol at the STI clinic. For sample collection, study participants had a cervical speculum examination to collect three cervical swabs. The first was for the rapid test, the second for the culture test, and the third as back-up. Participants were treated according to the results of the gold standard test (culture).

The reference standard test for isolation and identification of *N gonorrhoeae* used in the study was culture using modified Thayer-Martin medium. Pre-made agar plates were stored at the clinic and inoculated with the cervical swab by a health professional immediately after collection. Inoculated plates were transported to the laboratory site in the same building as the clinic as soon as possible in accordance with the standard operating procedures. Inoculated plates were incubated at 35–36℃ in a humid atmosphere (70% humidity) containing 5% carbon dioxide (CO_2). The plates were checked for bacterial colonies at 24 and 48 hours. Colonies resembling those of *N gonorrhoeae* were confirmed using the oxidase, catalase and sugar oxidation tests.[15]

The GC OIA rapid test detects a unique target antigen found in all strains of *N gonorrhoeae*, and was done on site. The cervical swab was placed in a reaction tube with an added reagent for extraction. The swab was discarded and two drops of the extract were pipetted from the first to the second reaction tube. Reagent 2, a neutraliser, was added to the reaction tube and the solution changed to red/pink before proceeding. Next, one drop of the extracted neutralised sample was put on the centre of the test surface. After 5 minutes, one drop of reagent 3, the conjugate, was added to the sample, then the test surface was vigorously washed twice with an interval between of 10 minutes. Upon completion, the test surface was examined under a bright light. An internal control dot is visible as a small blue dot in the centre upon completion of a test. A negative test shows only this control dot, while a positive test result shows the internal dot inside a reactive circle.

Rapid test results were read independently by two trained staff members. A technical supervisor was trained to supervise the pilot run of the rapid test and the routine activities involved in rapid test evaluation, including patient recruitment, interviews, specimen collection, labelling, storage, and transport of samples to the laboratory site. The technical supervisor also ensured that laboratory staff were blinded to the concurrent rapid test results. Sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) were estimated for the GC OIA test on cervical specimens compared to culture. Ninety-five per cent confidence intervals (95% CI) were calculated according to the formula $1.96\sqrt{p(1-p)/n}$, where p is the point estimate, and n the number of subjects positive (for sensitivity) of negative (for specificity) by the gold standard.[16]

RESULTS

Age of participants ranged between 18–55 years. The mean age of women in the study was 25.3 years, and the median 24 years. The prevalence of gonococcal infection in study participants was 15% (50/326). In the asymptomatic subgroup, the prevalence of gonococcal infection was 17.7% (25/141), compared with 13.5% in the symptomatic group (25/185) ($p = 0.3$). The highest prevalence of infection (23.5%) was found in the 20–29 year age group. The sensitivity, specificity, PPV and NPV values and 95% CI were 60% (46.4% to 73.6%), 89.9% (86.2% to 93.6%), 55.6% (42.4% to 68.8%), and 92.6% (89.5% to 95.7%), respectively (table 1).

Four members of staff performed the NG Biostar test. All found the test instructions easy or very easy to follow, and the performance of the test and interpretation of results easy or very easy. Three of the four were able to obtain results within 30 minutes of receiving the specimen; 98.8% of study participants were prepared to wait up to 1 hour for their results.

DISCUSSION

This study investigates the performance and acceptability of a rapid test for *N gonorrhoeae* in the setting of an STI clinic in Brazil, a developing country with a high prevalence and incidence of STI. Syndromic management protocols have proved to be the most effective for STI treatment in the developing world. They have proved successful in reducing STI transmission, educating patients, and treating easily cured STIs that might otherwise lead to higher HIV transmission risk[6 10 17] However, the syndromic approach flowchart does not distinguish between vaginal discharge and cervical infection, leading to considerable over-treatment for gonorrhoea and chlamydial infection.[8 9] With the cost of antibiotics and increasing resistance of *N gonorrhoeae*, more accurate methods are needed to definitively diagnose gonococcal infection. Although the sensitivity of the NG Biostar test is suboptimal, its high NPV in this population suggests that it could be useful as a means of reducing over-treatment for *N gonorrhoea* in women presenting with vaginal discharge

If rapid tests for *N gonorrhoeae* infection become widely available, they could also be used to screen asymptomatic women in outreach programmes. However, the sensitivity of the NG Biostar test may be even lower in asymptomatic women. Moreover, the need for a speculum examination limits its usefulness in this context.

The use of the NG Biostar rapid test for gonococcal infection test was assessed by clinic staff as easy to understand and perform. The results were also regarded as easily interpreted and are ready well within the time that most patients are willing to wait in the clinic for diagnosis. The sensitivity (60%) and PPV (55.6%) are less than ideal for practical use to detect gonococcal infection. However, failure to return to the clinic for

Table 1 Comparison of test results on cervical swabs

	Modified Thayer-Martin culture		
NG Biostar	Positive	Negative	Total
Positive	30	24	54
Negative	20	248	268
Total	50	276	326

laboratory test results means many infected patients do not receive treatment and continue to disseminate the infection to their sexual partners while increasing their risk of developing pelvic inflammatory disease. High-risk women will also lower their susceptibility to HIV infection if they receive treatment during their initial visit.

Modelling studies have shown that if the rapid test sensitivity is more than 60%, gonoccocal infections are likely to be more efficiently averted through their use than through the use of a laboratory-based test.[9] Even a rapid test with suboptimal sensitivity can improve rates of treatment compared to a gold standard test that requires return visits for patients to receive results and treatment.

It is clear that setting and population characteristics are of great importance in considering the use of a rapid test. Given the antigenic variation characteristic of *N gonorrhoeae*, and the fact that the target antigen for this assay is not known to the scientific community, the performance of this assay may vary in different populations, and over time. While the study staff found the test easy to perform and interpret, the performance of any diagnostic test may decline when it is used under routine conditions rather than in a research project.

Point-of-care tests are especially useful in high risk populations such as sex workers, in whom early treatment will prevent a substantial number of new infections. A rapid test that could be used in outreach programmes as well as in clinics would be especially valuable in these populations. Cost-effectiveness studies of rapid point-of-care tests for *N gonorrhoeae* infection compared to the syndromic approach should be carried out to assess their value in STI diagnosis and treatment in developing nations.

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